Neonatal Thyroxine Level and Perchlorate in Drinking Water

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Environmental contamination of drinking water has been observed for perchlorate, a chemical able to affect thyroid function. This study examines whether that exposure affected the thyroid function of newborns. Neonatal blood thyroxine (T_4) levels for days 1 to 4 of life were compared for newborns from the city of Las Vegas, Nevada, which has perchlorate in its drinking water, and those from the city of Reno, Nevada, which does not (detection limit, 4 μg/L [ppb]). This study is based on blood T_4 analyses from more than 23,000 newborns in these two cities during the period April 1998 through June 1999. No difference was found in the mean blood T_4 levels of the newborns from these two cities. Drinking water perchlorate levels measured monthly for Las Vegas ranged during this study period from non-detectable for 8 months to levels of 9 to 15 ppb for 7 months. Temporal differences in mean T_4 level were noted in both cities but were unrelated to the perchlorate exposure. This study was sufficiently sensitive to detect the effects of gender, birth weight, and the day of life on which the blood sample was taken on the neonatal T_4 level, but it detected no effect from environmental exposures to perchlorate that ranged up to 15 μg/L (ppb).

Perchlorate competitively inhibits the uptake of iodide into the thyroid gland.[1] The pharmacology of perchlorate was well described by Wolff.[2] Blockade of iodine uptake leads to increased serum thyrotropin levels and decreased blood thyroxine (T_4) levels. Perchlorate is used medically to treat hyperthyroidism of various pathogeneses (eg, Graves' Disease, amiodarone toxicity) at doses of 900 mg/day or less. Studies of workers showed no effect on thyroid function at 34 mg/day.[3] Studies of subjects ingesting oral perchlorate doses of 10 mg/day for 2 weeks showed no effect on thyroid function despite a demonstrated partial inhibition of iodide uptake.[3a] Perchlorate has been shown in guinea pigs to cause a fetal goiter at dosages (1% in water) that did not cause a maternal goiter.[4]

Several public water supplies in California and southern Nevada contain perchlorate in the 4- to 16-ppb concentration range. Newborns are screened for metabolic diseases in hospitals and doctor's offices in a mandatory state-run program. These programs include the measurement of neonatal T_4 levels as a screening procedure for congenital hypothyroidism. An analysis of the data from these programs for California and Nevada has demonstrated that the incidence of congenital hypothyroidism among children born in areas with perchlorate in drinking water did not differ from the incidence in perchlorate-free areas.[5] The present study supplements this observation by examining for newborns in Nevada whether there is an effect on

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neonatal thyroid status as determined by the neonatal T_4 level associated with the presence of perchlorate in the drinking water.

Materials and Methods

The drinking water supply for Clark County, Nevada, and its largest city, Las Vegas, comes from a part of Lake Mead that is contaminated with perchlorate. In some months, the level is below the detection limit of 4 ppb (μg/L); in other months the concentration is measured in the range of 4.1 to 15 ppb. This is the only documented source of perchlorate contamination of a public water supply in Nevada. The city of Reno, the second largest city in Nevada, is 450 miles away in the foothills of the Sierra Nevada mountains and has an independent source of drinking water that has no evidence of perchlorate contamination.

For the period April 1998 through June 1999, we compared the mean monthly T_4 levels of newborns in two urban areas in Nevada, one (Las Vegas) with perchlorate and one (Reno) without perchlorate in the drinking water supply. The population of Las Vegas is about 3 times that of Reno. The study cohorts consist of all newborns who had blood samples submitted within 4 days of birth, who had birth weights between 2500 and 4500 grams, and who had not been admitted to a Neonatal Intensive Care Unit by the time of blood sample collection. Demographic data on the newborns included date of birth, date of sample collection, gender, birth weight, birthplace, and office address of each newborn's pediatrician.

Outcome Variable

The outcome of interest was neonatal blood T_4 level, as measured by the Oregon State Public Health Laboratory for the Nevada State Health Department. All states measure neonatal thyroid status (either T_4 or serum thyrotropin) in heel blood obtained in the hospital during the first few days of life, as part of the legally mandated neonatal metabolic diseases screening program. In Nevada, over 80% of newborns also have a later blood sample submitted for T_4 analysis from the physician's office at a follow-up visit.

The Oregon State Public Health Laboratory provides these analyses for the Nevada State Health Division program, including births in both Las Vegas and Reno. The laboratory used a radioimmunoassay method[6] to analyze T_4 levels in the congenital hypothyroidism screening program. The blood T_4 data were analyzed as a continuous variable. T_4 levels depend on the stability of the particular assay method in a laboratory. The Oregon State Public Health Laboratory established stabilization of their T_4 assay most recently in March 1998, thus defining the time period of observation for this study as beginning in April 1998.

Exposure Variable

The exposure factor of primary interest was perchlorate in drinking water, which occurred in Las Vegas and not in Reno. Lake Mead is the sole source for the public water supply of Las Vegas. Perchlorate in the intake of water to the city of Las Vegas occurred when the turbulent conditions of Lake Mead overcame the thermal stratification of the lake and thus presented contaminated water to the intake of the water supply. Monthly measurements of the perchlorate levels in Las Vegas finished water have been made since July 1997 by the Southern Nevada Water Authority, using a method with a detection limit of 4 ppb (μg/L) developed by the California Department of Health Services, Sanitation and Radiation Laboratory in April 1997.[7] Perchlorate was detected in the Las Vegas drinking water during 7 of the 15 months in this study period. Analyses have been performed comparing the T_4 levels of the children born in the 7 study months in which perchlorate was detected in the Las Vegas water supply (time period A) and those born in the 8 study months in which perchlorate was not detected in the Las Vegas water supply (time period B). Perchlorate was also detected in the Las Vegas water supply during the 9 months before the study period.

The water supply in Reno had no connection with Lake Mead or the sources of perchlorate in Lake Mead. Instead, Reno derives 80% of its supply from the high mountains via Lake Tahoe and the Truckee River and 20% from local wells in the Reno area. Tests of the water sources for Reno, using the same laboratory method, detected no perchlorate.[8]

Statistical Methods

The distributions of demographic variables of the newborns of the two cities were compared using a chi-squared test for categorical variables and a *t* test for continuous variables. The mean T_4 levels between the 17,308 newborns in Las Vegas and the 5882 newborns in Reno were compared in a univariate analysis, both crude and stratified by time period. A multivariate analysis was performed with T_4 level as the outcome variable; city and time period as the main effect variables; and gender, birth weight, and age at time of sample collection as the covariates. An interaction term between city and time period was included in the model as a marker for perchlorate exposure. All statistical tests were two-sided, and the cutoff probability for a type I error was 0.05. All analyses used Stata statistical software (version 5.0).

Initial analyses used the T_4 levels of the blood samples collected in the hospital up through each newborn's fourth day of life and the perchlorate level in the Las Vegas drinking water during the month of birth, expressed in μg/L. Subsequent analyses used the same T_4 levels and the cumulative perchlorate levels, expressed in μg/L-months, for either the 9 months of pregnancy or the time period equivalent to the first trimester of pregnancy. Additional analysis used the T_4 levels of the bloods submitted

TABLE 1
Demographic Comparisons Between Las Vegas and Reno, Nevada of Newborns Screened With T_4 Test (April 1998–June 1999)

	Las Vegas (n = 17,308)	Reno (n = 5,882)	P Value
Gender (% male)	51.3	51.1	0.779
Mean birth weight (G)	3,379	3,365	0.033*
Mean age at time of sample collection (days)	1.20	1.45	0.0000*

* Differences between the pairs are statistically significant at less than P = 0.05 level.

TABLE 2
Analytic Comparisons of Mean Monthly Neonatal T4 Levels of Las Vegas and Reno, Nevada (April 1998–June 1999), Directly and Stratified by Time Periods

City-Specific Mean T_4 Levels (μg/dL)	Place		
	Las Vegas	Reno	P Value
Total sample	17.11	17.12	0.901
Stratified by time period*			
A (7 months)	16.78	16.77	0.876
B (8 months)	17.37	17.41	0.692
Difference between A and B	−0.59	−0.64	0.699

* During the 15-month study period, time period A was defined as a combination of those months at which perchlorate levels were detected in the drinking water in Las Vegas, Nevada, whereas time period B included the remaining months at which perchlorate level were not detected in the drinking water in Las Vegas, Nevada.

TABLE 3
Multivariate Analysis of the Effects of City, Time Period and Their Interaction on Neonatal T_4 Levels After Adjusting for Gender, Birth Weight, and Age at Time of Sample Collection[a]

	Difference in Mean T4 (μg/dL)	95% CI[b]	P Value
Main effects and their interaction			
City (Las Vegas vs Reno)	−0.069	−0.232–0.094	0.407
Time period (B vs A)*	0.595	0.484–0.706	0.000
Interaction (city and time period)	0.021	−0.198–0.240	0.850
Control variables			
Gender (female vs male)	0.727	0.632–0.823	0.000
Birth weight (per 1000/G)	0.850	0.735–0.964	0.000
Age at time of sample collection†			
1st vs 4th day	−1.275	−1.552–0.999	0.000
2nd vs 4th day	0.408	0.206–0.610	0.000
3rd vs 4th day	0.758	0.538–0.978	0.000

[a] Based on a cohort of 23,190 newborns delivered in Las Vegas, and Reno, Nevada, during the 15-month period of April 1, 1998 and June 30, 1999.

[b] CI, confidence interval;

* During the 15-month study period, time period A was defined as a combination of those months at which perchlorate levels were detected in the drinking water in Las Vegas, Nevada, whereas time period B included the remaining months at which perchlorate levels were not detected in the drinking water in Las Vegas, Nevada.

† The variable was treated as a categorical variable in the multivariate

from the physician's offices and examined the differences in the mean T_4 level of the infants of the two cities by the age of the newborn in days for the first 60 postnatal days.

Results

There were 23,305 newborns from Las Vegas and Reno who met the inclusion criteria during the 15-month period between April 1, 1998 and June 30, 1999, and 23,190 of them (99.5%) had valid neonatal T_4 measurements. The values for 115 newborns were excluded either because of unsatisfactory blood samples (n = 113) or invalid T_4 measurement (n = 2). The final study cohort consisted of 17,308 newborns from Las Vegas and 5882 from Reno.

Table 1 displays the distributions and corresponding P values of three potential confounding factors for the two cities. The newborns from Las Vegas and Reno did not differ in gender distribution but did in average birth weight and age in days at time of sample collection. The percentage of male infants in Las Vegas resembled that in Reno, 51.3% versus 51.1% (P = 0.779). The average birth weight of 3379 G in Las Vegas exceeded the average birth weight of 3365 G in Reno (P = 0.033). The mean number of days between the day of birth and the day of sample collection also differed significantly between Las Vegas and Reno, 1.20 versus 1.45 days ($P < 0.001$).

Figure 1 displays, for the 15-month period of April 1998 through June 1999, the monthly mean neonatal T_4 values for Las Vegas and for Reno. These values are about 17 μg/dL, with little difference between the two cities and little variation across the time period. No particular seasonal variation is evident. Figure 1 also displays the perchlorate level in the Las Vegas drinking water for each study month. Perchlorate was detected in the drinking water in 7 of the 15 months, ranging between 9 and 15 ppb (μg/L), but not in the other 8 months. The limit of detection was 4 ppb. Figure 1 shows no covariation between the perchlorate level in the Las Vegas drinking water and the mean monthly neonatal T_4 level in either Las Vegas or Reno.

Figure 1 shows that some months lacked detectable perchlorate in the Las Vegas water, which allowed the introduction of a second exposure-related variable. We designated time

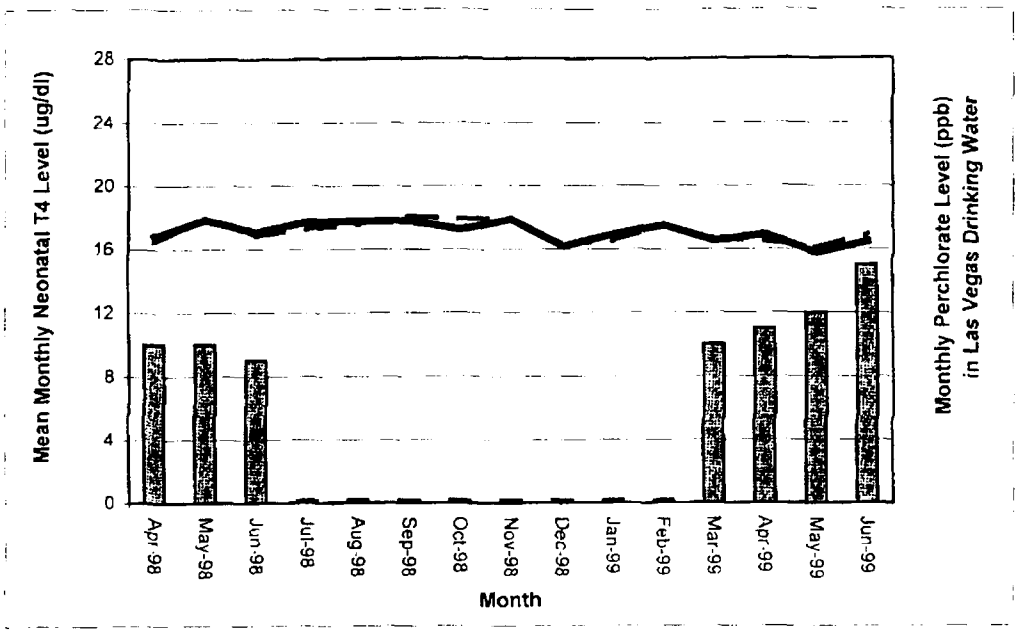

Fig. 1. Temporal presentation of mean monthly neonatal T4 (μg/dl) in Reno (dashed line) and Las Vegas (bold line) and monthly perchlorate (ppb) level (columns) in Las Vegas drinking water. Detection limit was 4 ppb (μg/L).

period A as the months with detectable perchlorate in Las Vegas water and time period B as the months without detectable perchlorate in Las Vegas water.

Table 2 shows that the mean T_4 level was 17.11 μg/dL in Las Vegas and 17.12 μg/dL in Reno (P = 0.901) in the analysis of the crude data. Stratification of the data by time period (period A and period B) showed no difference between the means of the two cities during either period A or period B (ie, no place effect). For each city, there was a difference in their mean T_4 levels for period A and period B (ie, a period effect). The period effect was similar in both cities (P = 0.699).

Table 3 displays the results of a multivariate analysis. As in the crude analysis, the multivariate analysis demonstrated no statistically significant difference in mean T_4 values with respect to place (ie, Las Vegas vs Reno; P = 0.407). However, it did show a significant difference with respect to period (ie, B vs A; P = 0.000). Because there was no significant interaction between place and period, the period effect could not be explained by the presence of perchlorate in only one of the two places. The above analysis was controlled for gender, birth weight, and age at time of sample collection, all of which were significant covariables (P = 0.000 for each).

The above analyses used the mean monthly T_4 level as the summary statistic to represent the T_4 distribution in either city for any particular month. This statistic represents the central tendency of the distribution. The lower tail (or extreme) of the distribution might also be used to represent the T_4 distribution in either city for any particular month. We examined this by comparing the 10th percentile levels for either city during periods A and B and found that the period effects in the two cities were indistinguishable. Thus, the two cities did not differ in their prevalence rates of low neonatal blood T_4 levels with respect to presence of perchlorate in the Las Vegas drinking water.

Perchlorate concentration data exist for Las Vegas for the 9 months preceding this study period. An estimate can thus be made on a monthly basis of the perchlorate exposure those newborns would have had during their full period of gestation. The Las Vegas newborns during this study period would have had perchlorate exposures that ranged between 9 ppb-months (μg/L-months) and 83 ppb-months (mean, 48 ppb-months); the Reno newborns during this period are presumed to have had 0 ppb-months. For each month, the difference between mean neonatal T4 levels of the two cities was examined, along with the cumulative perchlorate exposure estimated to have been experienced by those newborns. Linear regression analysis (Fig. 2) showed no evidence of an association (slope = −0.0003; R^2 = 0.002). Similarly, when estimated exposure during the first trimester of pregnancy was used as the exposure variable (0.0–36 ppb-months; mean, 20 ppb-months), linear regression analysis also showed no evidence of

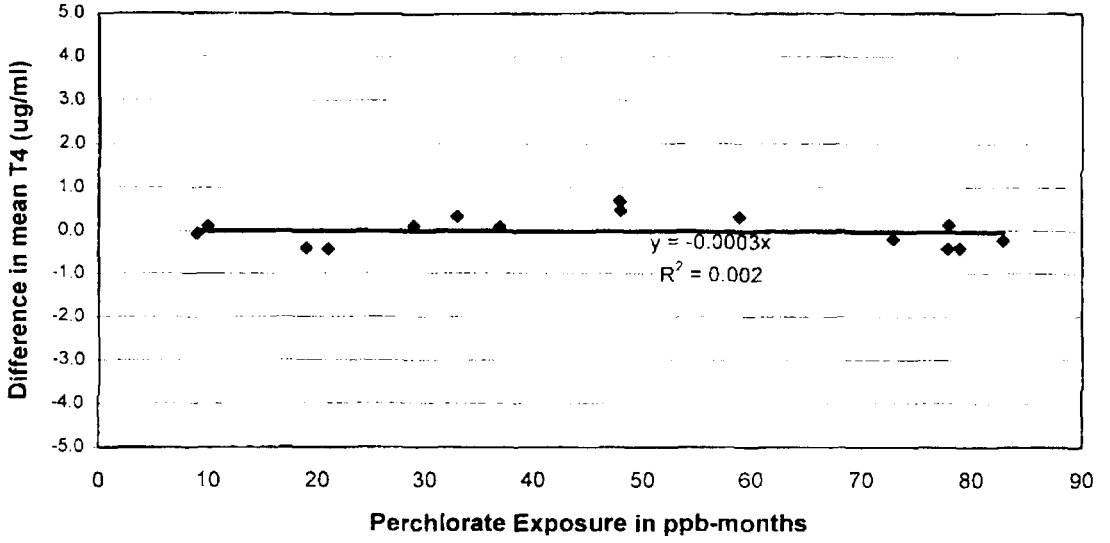

Fig. 2. Difference in mean neonatal T4 levels (μg/dl) between Reno and Las Vegas by estimated 9-month cumulative perchlorate exposure in Las Vegas.

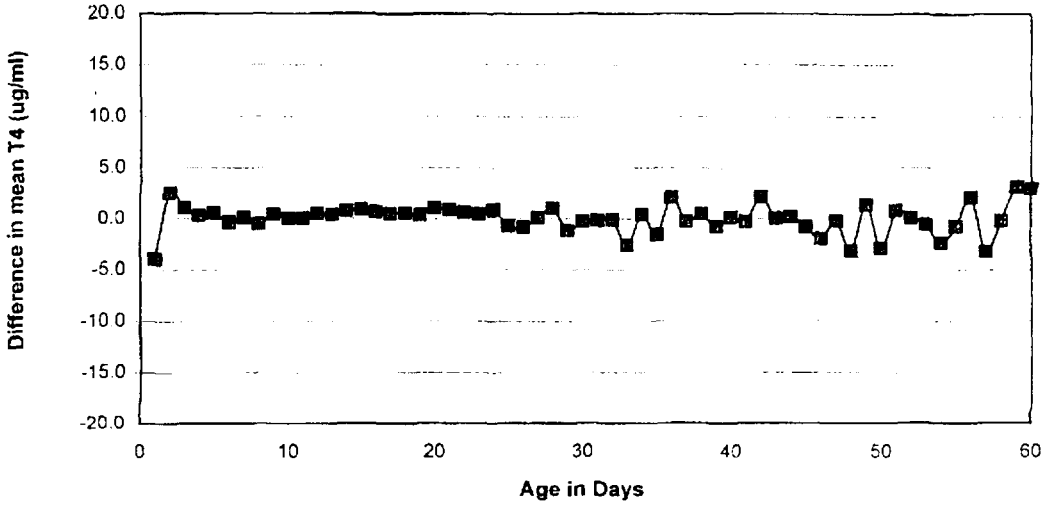

Fig. 3. Difference in mean T4 levels (μg/dl) between Reno and Las Vegas by age in days (1–60).

an association (slope = 0.0008; R^2 = 0.002).

Most newborns in Nevada (82%) have a blood specimen taken in the physician's office for a follow-up T_4 measurement, 99% of which are taken within the first 60 postnatal days. Figure 3 shows on a daily basis the difference between the mean T_4 levels of the infants from Reno and those from Las Vegas. Throughout the first 60 days, there seems to be no systematic difference between the mean T_4 levels in the two cities.

Discussion

The primary purpose of the study design was to compare the neonatal T_4 levels in Las Vegas, where perchlorate was present in the drinking water, with the neonatal T_4 levels in Reno, where perchlorate was not present. This comparison showed that perchlorate at environmental levels of up to 15 ppb did not affect neonatal T_4 levels during the study period. Our study design with respect to T_4 levels eliminated city/rural confounders by restricting the study populations to urban births, inter-laboratory variation by using data from the same laboratory, and seasonal effects by taking contempora-

neous data. Our analysis demonstrated that it was necessary to control for the known confounders of birth weight and age at time of blood sampling. We found no difference in the mean T_4 levels between the two cities, though we did find a period effect in the multivariate analysis. Because the period effect was essentially the same in Las Vegas and in Reno, it could not be explained by the presence of perchlorate only in the Las Vegas drinking water. These findings demonstrate the importance of having a comparison population. The most probable explanation relates to minor temporal changes in laboratory procedures or materials or seasonal variation. Early treatment of congenital hypothyroidism has been found to prevent intellectual loss in all but those with very low neonatal T_4 levels. Tillotson et al[9] demonstrated in Great Britain that the mean IQ of children with (T_4) values above the threshold of 42.8 *nmol/l* (3.34 μg/dl) was close to that of controls of similar social class. None of the newborns in this study was found to have a T_4 value below 3.70 μg/dl.[9]

Data do not exist on how many of the 23,190 women consumed public water during their pregnancy. However, there is no reason to believe that that consumption would have changed during the time period of this study. Therefore, it is assumed that the temporal pattern of perchlorate concentration in the Las Vegas drinking water mirrors the temporal pattern of perchlorate consumption by the maternal population in Las Vegas. The Environmental Protection Agency has traditionally assumed that each adult consumes 2 L of water per day, without making any specific distinction as to whether or not they are pregnant. We used the same 2-L per day assumption in our calculations. Any variation from that assumption, however, should be uniform over the time period of our study and should not cause a differential effect on the analysis. Nonetheless, dose reconstruction can be performed by using these assumptions. The cumulative exposures ranged from 0.9 to 4.2 mg with a mean of 2.2 mg during the pregnancy (ie, 2.2 mg perchlorate per gestational period), and from 0 to 1.7 mg with a mean of 0.9 mg during the first trimester.

There is little literature on the effects of perchlorate exposure during human pregnancy. Our earlier article[5] demonstrated no increase in the incidence of congenital hypothyroidism in areas of California and Southern Nevada with perchlorate in the drinking water, also at levels up to 16 ppb. Neither our earlier nor the current article showed any effect from low environmental contamination levels with exposures of up to 32 μg/day. On the other end of the fetal exposure spectrum is the experience with perchlorate as a therapeutic agent. In 1960, Crooks and Wayne[10] reported in *Lancet* their results from treating 12 women with thyrotoxicosis of pregnancy with potassium perchlorate at exposures of 600 to 2000 mg/day. They noted that "one of the infants had a very slight enlargement of the thyroid gland which disappeared within 6 weeks. The remainder showed no abnormality of any kind."

Although no analysis can rule out a small effect of these perchlorate exposures on neonatal T_4 levels, the current study does rule out a significant effect. Because this study found that mean blood T_4 levels differed significantly with sex, birth weight, and age at time of sample collection, it likely had sufficient statistical power to detect any significant change induced by these perchlorate exposures. We conclude that perchlorate in drinking water at a level of up to 15 ppb had no detectable effect on neonatal T_4 levels in this population.

Acknowledgment

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